DOI:
10.1016/j.mri.2015.12.021

Document Version
Peer reviewed version

The final article has a DOI of: 10.1016/j.mri.2015.12.021

Title: Age-effects in white matter using associated Diffusion Tensor Imaging and Magnetization Transfer Ratio during late childhood and early adolescence

Luciana Monteiro Moura[a,b,e,*], Matthew Kempton[e], Gareth Barker[e], Giovanni Salum[c,g]. Ary Gadelha[a,b], Pedro Mario Pan[a,b], Marcelo Hoexter[f], Marco Antonio Gomes Del Aquilla[a,b], Felipe Almeida Picon[c,g], Mauricio Anés[c], Maria Concepcion Garcia Otaduy[f], Edson Amaro Jr.[f], Luis Augusto Rohde[c,g], Philip McGuire[e], Rodrigo Affonseca Bressan[a,b], João Ricardo Sato[d], Andrea Parolin Jackowski[a,b]

[a] Department of Psychiatry, Federal University of Sao Paulo, Sao Paulo, Brazil

[b] Interdisciplinary Lab of Clinical Neurosciences (LiNC), Federal University of Sao Paulo (UNIFESP), Sao Paulo, Brazil

[c] National Institute of Developmental Psychiatry for Children and Adolescents, CNPq, Brazil

[d] Center of Mathematics, Computation and Cognition, Universidade Federal do ABC, Santo Andre, Brazil

[e] Institute of Psychiatry, Psychology and Neurosciences; King's College, London, UK

[f] Laboratory of Magnetic Resonance in Neuroradiology, LIM-44, Institute and Department of Radiology, University of Sao Paulo, Sao Paulo, Brazil

[g] Department of Psychiatry, Hospital de Clínicas de Porto Alegre, Federal University of Rio Grande do Sul, Brazil

*Corresponding author

Luciana Monteiro de Moura, PhD**,** Department of Psychiatry, Federal University of São Paulo, Rua Borges Lagoa, 570 1°Andar, CEP: 04039-032, São Paulo, Brazil, telephone number: +55(11) 5576-4845 Email: lummoura@gmail.com

ABSTRACT

In the last decade, several studies have described the typical brain white matter maturation in children and adolescents. Diffusion tensor imaging (DTI) is the most frequent MRI technique used to investigate the structural changes across development. However, few previous studies have used the magnetization transfer ratio (MTR), which gives a closer measure of myelin content. Here, we employed both techniques for the same sample of 176 typically developing children from 7 to 14 years of age. We investigated the associations between DTI parameters and MTR measure, to assess the myelination in the brain in development. Secondly, we investigated age-effects on DTI parameters (fractional anisotropy, axial, radial and mean diffusivities) and MTR. No significant correlations between MTR and DTI parameters were observed. In addition, a significant age-effect was detected for DTI data but was not visible for MTR data. Thereby, changes in white matter at this age might be primarily correlated with microstructural changes.

Keywords: magnetization transfer ratio, diffusion tensor imaging, normal brain development, childhood, adolescence

DTI - diffusion tensor imaging; antTHR – anterior thalamic radiation; FA - fractional anisotropy; MD – mean diffusivity; MTR – magnetization transfer ratio; CG – cingulum gyrus; CGH cingulum (hippocampus) FMj - forceps major; FMn – forceps minor; infFO – inferior fronto occipital fasciculus; infLF - inferior longitudinal fasciculus; supLF – superior longitudinal fasciculus; supLFtemp – superior longitudinal fasciculus (temporal part); UF – uncinate fasciculus;

1. INTRODUCTION

Defining typical trajectories for the maturation of brain's circuitry is a crucial step to contrast typical and abnormal development[1], especially during childhood and adolescence, when many psychiatric disorders have their onset[2]. Since 1994 the tensor model has been used in diffusion imaging to infer structural connectivity[3]. During this period, several studies have investigated the exact correlation between microstructure and diffusion tensor imaging (DTI) parameters [4]. Diffusion imaging is designed to estimate effective scalar diffusivity of water molecules[5]. Mean diffusivity (MD) parameter is an almost invariant measure , and anisotropy indices, such as fractional anisotropy (FA), aim to characterize directionality according to the tensor model[6,7]. Despite many efforts in this direction, the relationship between DTI parameters and myelination remains unclear[8] since the relation between DTI parameters and white matter compartmental microstructure is still not well understood[4].

Given the intrinsic limitations of MRI acquisition and tensor model interpretation, combining different MRI techniques may help to reveal the microstructure of the white matter tracts. One particularly attractive direction is to use magnetization transfer (MT). MT estimates the signal that comes specifically from macromolecules[9]. Given that T2 relaxation time of macromolecules is too short to be directly measured with MRI, MT contrast imaging may be used to indirectly detect the signal coming from bound water linked to these molecules. The application of an off-resonance radiofrequency pulse preferentially saturates immobile or restricted water protons associated with macromolecules, such as myelin or the axonal membrane[9,10] which then transfer energy to surrounding protons in free water. The ratio of this energetic transference can then be quantified. The more macromolecules (including myelin) content is present in the voxel, the lesser is the signal after MT[9,11], which configures the magnetization transfer ratio (MTR). Brain MT measurements in the literature have been acquired in a variety of ways. Therefore, absolute MTR values vary considerably from study to study[12]. Even so, it is a very useful and reliable technique in studies of healthy human brain[13,14] showing consistency with myelin content[15,16] and only secondary correlation with axonal count[16].

A deeper investigation of how white matter changes across childhood and adolescence is also fundamental to the understanding of parallel maturation patterns in grey matter. Microstructural changes in white matter are

associated with changes in cortical grey matter regions[17,18], but cannot be fully explained by white matter maturation in the underlying regions as measured by volumetric analyses or DTI[19]. Cortical thinning is seen as a maturational marker, especially in adolescence. Multiple hypotheses have been elaborated in order to explain this thinning: pruning of synapses, axons, and dendrites[1], a consequence of partial volume effects[20] such as myelination[1,20] or enlargement of axonal caliber[8,21]. Moreover, doubts remain about the possibility of inferring myelination from DTI, but it is still one of the most used MRI techniques in the literature to study white matter and it is frequently assumed to correlate with myelination. Conversely, anisotropy measured by FA could be affected by axon diameter, packing density[8,22] and intra-voxel coherence[23].

As there still remains a gap in the literature, this study firstly aims to understand correlations among DTI measures and also between them and the MTR measure. This approach could help disentangling possible sources of confusion in both techniques regarding the myelin content of a voxel. Using information derived from ROIs restricted to the center of the tracts, we propose a new methodological approach for MTR extraction, since looking at the center of the largest tracts can be a reliable approach[24]. Therefore, using the same approach for both MRI techniques allows us to reach a better comparison between measures. As a second aim, we examine age-effects on white matter through DTI and MTR measures.

2. METHODS

2.1. Participants

This sample was obtained from a large school-based community study investigating psychiatric and neurocognitive aspects, through genetics and neuroimaging[25]. From a total of 249 children without DSM-IV mental disorders, confirmed using the Diagnostic and Well-Being Assessment (DAWBA)[26], 176 were selected for which the images obtained were free of artifacts (i.e., motion, spikes) in both modalities (DTI and MTR[1]). Their age ranged from 7 to 14 (88 males/88 females). The estimated IQ was obtained using the Wechsler Intelligence Scale for Children, 3rd edition (WISC-III)[27], and Brazilian norms[28] were adopted. Children were partitioned into two groups according to the site of MRI scanning acquisition (Sao Paulo and Porto Alegre). The demographics of groups are displayed in Table 1.

The ethics committee of the University of Sao Paulo approved the procedures of this study. All parents provided written consent and all children provided verbal (or when possible, written) assent.

The socioeconomic classification (Brazilian rating scale - ABIPEME)[29] of the whole group was distributed as 4.4% very low (E and D classes), 65.9% medium (C and B classes) and 29.5% high (A class).

[1] including simultaneously high quality image MTon and MToff

2.2. MRI acquisition

MRI scanning was performed at two centers both using GE Signa 1.5-T MR systems Center (GE Healthcare, Milwaukee, WI, EUA): Images from 86 participants were acquired in a Signa HD scanner at Porto Alegre Site and images from 90 participants were acquired in a Signa HDX scanner at Sao Paulo.

All children underwent high-resolution axial T1 sequence (3D FSPGR sequence with NEX=1, FOV=24.0 x 18.0 cm, flip angle=15 degrees, TE=in phase 4.2 ms, TR=10.91 ms, matrix size= 256x192, slice thickness=1.2 mm, yielding 160 slices). An axial brain MR-DTI sequence was acquired (spin echo diffusion weighted EPI): b=800 s/mm2, 15 non-collinear directions, TE = 99 ms, TR = 11600 ms, matrix size = 128 x 128, NEX=2, FOV= 24.0 cm, slice thickness= 3 mm/without gap, yielding 47 slices). All images were visually inspected and no artifacts (i.e. motion, spikes) were detected on any of the images. Magnetization transfer ratio (MTR) images were also obtained in a dual acquisition with (MTon) and without (MToff) with an MT saturation pulse following the protocol: 3D Gradient Echo, NEX=1, FOV=24.0 x 18.00 cm, flip angle=12 degrees, TE= 4 ms, number of echoes=1, TR=27/28 ms, matrix size= 256x256, slice thickness=3.0 mm, yielding 50 slices.

2.3. Magnetization transfer image processing

We developed a processing pipeline for MTR data using FSL 4.1.9. For each subject the acquired MTon image was co-registered to the MToff image using FSL FLIRT, applying a rigid body transform. Following co-registration, an MTR percentage image (MT%) was subsequently calculated applying the equation 100(MToff – MTon)/MToff. To perform voxelwise analyses the MTR% image was required to be normalized to MNI space, however direct spatial normalization is problematic as the MTR data does have the same contrast as a standard T1 MNI template. Thus the MToff image (which is in the same space as the MTR% image) was initially coregistered to the T1 FSPGR image. The T1 FSPGR image was skull stripped using FSL BET and spatially normalized to MNI space, and the same transformation was applied to the MTR% image to ensure the MTR% image was in MNI space. To reduce inconsistencies due to nonlinear distortions we chose a non-linear normalization, the FSL FNIRT.

2.4. Diffusion tensor imaging post-processing

FA maps were generated according to the steps of the FSL platform software version 4.1.9[24]. After that, the registration was made using Tract-Based Spatial Statistics (TBSS) with inter subject alignment (-n flag) in order to

identify the most representative FA map of the sample. This representative reference image was then used as a target to non-linearly register every subject's FA image. Subsequently, the whole aligned dataset was normalized using an affine transformation into a MNI152 standard space (1 mm^3). The average of the aligned FA images was merged into a single 4D mean FA image. A mean FA skeleton was then derived from all aligned FA images, generating a single 4D mean FA skeleton image (a group FA skeleton), and the most relevant tracts from the spatially normalized FA map of each subject were projected onto this skeleton using a threshold of 0.2[24]. Nonlinear warps and skeleton projection were also applied to mean diffusivity (MD), axial diffusivity (AD) and radial diffusivity (RD).

Specific matrices of contrast were created using the FSL FEAT tool in order to conduct statistical analysis using the general linear model (GLM); age, acquisition site and gender were entered as covariates. Voxelwise statistical analyses were performed using the FSL Randomise tool; Monte Carlo permutation-based inferences were made on unsmoothed statistical maps applying 10000 permutations, family-wise error (FWE) corrected Threshold-Free Cluster Enhancement (TFCE)[30] was obtained at $P < 0.01$.

2.5. Regions of Interest (ROIs)

Using the statistical software 'R' we extracted mean FA values from the 4D skeleton for each subject. Twenty tracts were identified using the JHU (John Hopkins University) atlas of white matter tractography[31,32]. In order to extract the mean values of MTR data, we used a 4D MTR file (as a result of MTR processing described in 2.3).

2.6. Statistical analyses

Correlations between measures

First we performed Spearman correlations investigating correlations within DTI measures using the extract means of each tract: FA/MD, FA/axial diffusivity, FA/radial diffusivity and in addition between FA/MTR. Secondly, we investigated possible correlations between all DTI means (FA, MD, axial and radial diffusivity) and MTR means. All *P* values were corrected by Bonferroni post hoc test.

Age-effects

(i) Whole-brain TFCE analysis using TBSS: we investigated first the cluster-wise analysis of FA, mean, radial and axial diffusivity values in TBSS (according to description 2.4);

(ii) *ROI mean values analyses:* secondly we performed a univariate analysis in GLM. Mean values of each tract were used as dependent variables, considering the following measures: FA, MD, axial, radial diffusivity and mean MTR. As fixed factor we used the age, distributed in three groups (7-8, 9-11, 12-14 years of age) to enhance power effect (demographics of groups is available in table S1, supplementary material), while gender and site were used as covariates. Statistical analyses were made using SPSS version 22.0.

3. RESULTS

Demographics

TABLE 1

Correlations between measures

Considering the mean value for each measure (FA, MD, axial, radial diffusivity and MTR) of each tract, the correlation analyses between: FA/MD showed a negative significant correlation at *P* level < 10^{-5}, except for forceps minor (FMn) p = 0.001 and superior longitudinal fasciculus temporal part left (SLFtemp L) that showed no significant correlation; FA/axial diffusivity showed both, positive and negative correlations, most of them were not significant at *P*

level < 0.05, except for the positive correlation related to SLFtemp right at *P* level $<10^{-5}$; FA/radial diffusivity showed negative significant correlations at *P* level < 10^{-5}, an exception for the forceps minor that showed no significant correlation. Values are available in the Table 2.

TABLE 2

No significant correlations were founded between DTI means (FA, MD, axial and radial diffusivity) and MTR means (followed by Bonferroni post hoc correction), values are available in the table S2 (supplementary material).

Age-effects

(i) Whole-brain TFCE analysis using TBSS:

Whole-brain TFCE analysis using TBSS (*P* < 0.01, FWE correction): as expected, FA showed a widespread (involving multiple tracts) positive correlation with age, meanwhile MD and radial diffusivity showed a similar opposite pattern with age (Figure 1). Although, axial diffusivity also displays a negative correlation with age, findings were restricted to small clusters which were more significant in the left hemisphere. Association with site of acquisition was found for Sao Paulo and Porto Alegre.

FIGURE 1

(ii) *ROIs mean values analyses:*

Evaluating age-affect in the three age groups (7 -8; 9-11; 12-14 years of age), followed by Bonferroni post hoc correction (Table 3), we found that: FA showed significant differences regarding all the tracts, except for forceps minor (FMn); mean and radial diffusivity showed differences considering most of the tracts; axial diffusivity only showed significant differences regarding cingulum right (CGL), superior longitudinal fasciculus right and left (SLF); MTR showed no significant difference between age groups.

TABLE 3

4. DISCUSSION

In agreement with previous studies, FA showed positive correlation with age, while mean, axial and radial diffusivities showed negative correlations with age[33-35]. When analyzing the DTI means extracted from ROIs of each tract, there were significant differences among the three age groups. Differences regarding FA, MD and radial diffusivity involved most of the tracts but only three of them were involved when considering the axial diffusivity. On the contrary, no significant differences were identified between groups when using

MTR measures. With respect to correlations within measures, FA was significantly correlated to mean and radial diffusivity but not to axial diffusivity. In turn, MTR did not correlate with any of the DTI measures. These findings point to a possible dissociation between DTI measures and MTR at this age. Thereby, changes in white matter could be more closely correlated to microstructural changes and only secondary to myelination.

Although MTR has been considered a more useful measure to track early white matter developmental changes[36], our results support a lack of age-effect on MTR which is consistent with an earlier stabilization of myelin content[37]. Despite this lack of correlation between MTR values and age, previous literature indicates that white matter volume continues growing through adolescence until adulthood [2][34,38] as well as FA[33,34,39]. Therefore the correlation between increase in volume, myelination process and microstructural changes in white matter[2] remains unclear.

The increase in FA that is age-related seems to be mainly driven by a decrease in mean and radial diffusivity, as a decrease in axial diffusivity is more restricted to few tracts. Therefore the directionality of the fibers seems to be already established at this age. Then, decreased radial diffusivity (which depicts decreased diffusion in the perpendicular axis) could points to changes in the surrounding environment of the fiber primarily affected by factors such as: increased spatial coherence[4,40]; membranes themselves[4]; increase in

packing density that is compatible with the negative age-effect on MD, allowing to infer that the amount of hindered diffusivity decreases[41]. In turn, myelination could play a secondary role. When looking to the correlations between measures, we have found significant correlations within the diffusion parameters but they do not correlate with MTR. This finding is in line with the lack of correlations between DTI parameters and another MT measure, the cross-relaxation[42].

The main limitations of our study are related to the intrinsic problem of comparing MTR studies. It is difficult to ensure the reproducibility of findings, given the wide range of acquisition parameters and protocols that have been used in literature and the different methodologies of registration and extraction in the post processing analysis (i.e. by brain region). Despite the lack of gold standard to study non-invasively myelination, MTR has been considered as a reliable index of myelination across studies. We also used a DTI acquisition with 15 directions that would only allow tractography of the major white matter tracts. The use of protocols with higher b values could be interesting to better understand issues related to the restrict diffusion environment. In addition, it is worth considering that due to the sample size, we might not have a sufficient power to detect small variations.

Our findings support the need of multimodal analysis for the best characterization of structural changes in the typical development of the white

matter. Although parameters of diffusion are subjected to the interference of myelin content, which provides a restrictive barrier that hinders diffusion in the bundle, these findings suggest that myelination has already stabilized at this age. How white matter structurally changes across development is a fundamental issue in the understanding of structural brain connectivity, and therefore the study of these changes contributes to the comprehension of the underlying structural mechanisms of cortical changes.

Acknowledgements

The opinions, hypotheses, conclusions, and recommendations of this study are the responsibilities of the authors and are not necessarily representative of the opinions of the funding agencies. The authors are grateful to the CAPES Foundation for the fellowship (Moura, L.M. 17930/12-0), the Sao Paulo Research Foundation–FAPESP (Sato, J.R. grant 2013/ 10498-6 and 2013/00506-1 and Jackowski, A.P. grant 2013/08531-5), and CNPq (Jackowski, A.P. grant 442026/2014-5), Brazil for funding this research. This study is from the National Institutes of Science and Technology for Developmental Psychiatry of Children and Adolescents (INPD) and is supported by CNPq (573974/2008-0) and FAPESP (2008/ 57896-8).

REFERENCES

[1] Tau GZ, Peterson BS. Normal development of brain circuits. Neuropsychopharmacology 2010;35:147–68. doi:10.1038/npp.2009.115.

[2] Paus T, Keshavan M, Giedd JN. Why do many psychiatric disorders emerge during adolescence? Nat Rev Neurosci 2008;9:947–57. doi:10.1038/nrn2513.

[3] Basser PJ, Mattiello J, LeBihan D. Estimation of the effective self-diffusion tensor from the NMR spin echo. J Magn Reson B 1994;103:247–54.

[4] Heidi Johansen-Berg Editor TEJB. Diffusion MRI: From quantitative measurement to in-vivo neuroanatomy. 2nd ed. London: Academic Press; 2014.

[5] Basser PJ. Inferring microstructural features and the physiological state of tissues from diffusion-weighted images. NMR Biomed 1995;8:333–44.

[6] Le Bihan D, Mangin JF, Poupon C, Clark CA, Pappata S, Molko N, et al. Diffusion tensor imaging: concepts and applications. J Magn Reson Imaging 2001;13:534–46. doi:10.1002/jmri.1076.

[7] Nagy Z, Weiskopf N, Alexander DC, Deichmann R. A method for improving the performance of gradient systems for diffusion-weighted MRI. Magn Reson Med 2007;58:763–8. doi:10.1002/mrm.21379.

[8] Paus T. Growth of white matter in the adolescent brain: myelin or axon? Brain Cogn 2010;72:26–35. doi:10.1016/j.bandc.2009.06.002.

[9] Henkelman RM, Stanisz GJ, Graham SJ. Magnetization transfer in MRI: a review. NMR Biomed 2001;14:57–64. doi:10.1002/nbm.683.

[10] Tofts P. Quantitative MRI of the Brain. Chichester, UK: John Wiley & Sons; 2005. doi:10.1002/0470869526.

[11] Engelbrecht V, Rassek M, Preiss S, Wald C, Mödder U. Age-dependent changes in magnetization transfer contrast of white matter in the pediatric brain. AJNR Am J Neuroradiol 1998;19:1923–9.

[12] Vavasour IM, Whittall KP, MacKay AL, Li DK, Vorobeychik G, Paty DW. A comparison between magnetization transfer ratios and myelin water percentages in normals and multiple sclerosis patients. Magn Reson Med 1998;40:763–8.

[13] Mehta RC, Pike GB, Enzmann DR. Magnetization transfer MR of the normal adult brain. AJNR Am J Neuroradiol 1995;16:2085–91.

[14] Levesque IR, Sled JG, Narayanan S, Giacomini PS, Ribeiro LT, Arnold DL, et al. Reproducibility of quantitative magnetization-transfer imaging parameters from repeated measurements. Magn Reson Med 2010;64:391–400. doi:10.1002/mrm.22350.

[15] Schmierer K, Scaravilli F, Altmann DR, Barker GJ, Miller DH. Magnetization transfer ratio and myelin in postmortem multiple sclerosis brain. Ann Neurol 2004;56:407–15. doi:10.1002/ana.20202.

[16] Schmierer K, Tozer DJ, Scaravilli F, Altmann DR, Barker GJ, Tofts PS, et al.

Quantitative magnetization transfer imaging in postmortem multiple sclerosis brain. J Magn Reson Imaging 2007;26:41–51. doi:10.1002/jmri.20984.
[17] Giorgio A, Watkins KE, Douaud G, James AC, James S, De Stefano N, et al. Changes in white matter microstructure during adolescence. NeuroImage 2008;39:52–61. doi:10.1016/j.neuroimage.2007.07.043.
[18] Giorgio A, Watkins KE, Chadwick M, James S, Winmill L, Douaud G, et al. Longitudinal changes in grey and white matter during adolescence. NeuroImage 2010;49:94–103. doi:10.1016/j.neuroimage.2009.08.003.
[19] Tamnes CK, Ostby Y, Fjell AM, Westlye LT, Due-Tønnessen P, Walhovd KB. Brain maturation in adolescence and young adulthood: regional age-related changes in cortical thickness and white matter volume and microstructure. Cereb Cortex 2010;20:534–48. doi:10.1093/cercor/bhp118.
[20] Paus T, Collins DL, Evans AC, Leonard G, Pike B, Zijdenbos A. Maturation of white matter in the human brain: a review of magnetic resonance studies. Brain Research Bulletin 2001;54:255–66.
[21] Jones DK, Knösche TR, Turner R. White matter integrity, fiber count, and other fallacies: the do“s and don”ts of diffusion MRI. NeuroImage 2013;73:239–54. doi:10.1016/j.neuroimage.2012.06.081.
[22] Takahashi M, Hackney DB, Zhang G, Wehrli SL, Wright AC, O'Brien WT, et al. Magnetic resonance microimaging of intraaxonal water diffusion in live excised lamprey spinal cord. Proc Natl Acad Sci USA 2002;99:16192–6. doi:10.1073/pnas.252249999.
[23] Beaulieu C. The basis of anisotropic water diffusion in the nervous system - a technical review. NMR Biomed 2002;15:435–55. doi:10.1002/nbm.782.
[24] Smith SM, Jenkinson M, Johansen-Berg H, Rueckert D, Nichols TE, Mackay CE, et al. Tract-based spatial statistics: voxelwise analysis of multi-subject diffusion data. NeuroImage 2006;31:1487–505. doi:10.1016/j.neuroimage.2006.02.024.
[25] Salum GA, Mogg K, Bradley BP, Gadelha A, Pan P, Tamanaha AC, et al. Threat bias in attention orienting: evidence of specificity in a large community-based study. Psychol Med 2013;43:733–45. doi:10.1017/S0033291712001651.
[26] Goodman R, Ford T, Richards H, Gatward R, Meltzer H. The Development and Well-Being Assessment: description and initial validation of an integrated assessment of child and adolescent psychopathology. J Child Psychol Psychiatry 2000;41:645–55.
[27] Wechsler D. WISC-III: Escala de Inteligência Wechsler para Crianças: Manual. 3rd ed. São Paulo: Casa do Psicólogo; 2002.
[28] Figueiredo V. Uma adaptação brasileira do teste de inteligência WISC-III. Brasília: Universidade Federal de Brasília; 2001.
[29] Almeida PM, Wickerhauser H. O critério ABA/ABIPEME: em busca de

uma atualização. São Paulo; 1991.
[30] Smith SM, Nichols TE. Threshold-free cluster enhancement: addressing problems of smoothing, threshold dependence and localisation in cluster inference. NeuroImage 2009;44:83–98. doi:10.1016/j.neuroimage.2008.03.061.
[31] Mori S, Wakana S, Nagae-Poetscher LM, Van Zijl PCM. MRI Atlas of Human White Matter. Elsevier; 2005.
[32] Wakana S, Caprihan A, Panzenboeck MM, Fallon JH, Perry M, Gollub RL, et al. Reproducibility of quantitative tractography methods applied to cerebral white matter. NeuroImage 2007;36:630–44. doi:10.1016/j.neuroimage.2007.02.049.
[33] Lebel C, Walker L, Leemans A, Phillips L, Beaulieu C. Microstructural maturation of the human brain from childhood to adulthood. NeuroImage 2008;40:1044–55. doi:10.1016/j.neuroimage.2007.12.053.
[34] Westlye LT, Walhovd KB, Dale AM, Bjørnerud A, Due-Tønnessen P, Engvig A, et al. Life-span changes of the human brain white matter: diffusion tensor imaging (DTI) and volumetry. Cereb Cortex 2010;20:2055–68. doi:10.1093/cercor/bhp280.
[35] Eluvathingal TJ, Hasan KM, Kramer L, Fletcher JM, Ewing-Cobbs L. Quantitative diffusion tensor tractography of association and projection fibers in normally developing children and adolescents. Cereb Cortex 2007;17:2760–8. doi:10.1093/cercor/bhm003.
[36] Wozniak JR, Lim KO. Advances in white matter imaging: a review of in vivo magnetic resonance methodologies and their applicability to the study of development and aging. Neurosci Biobehav Rev 2006;30:762–74. doi:10.1016/j.neubiorev.2006.06.003.
[37] Perrin JS, Leonard G, Perron M, Pike GB, Pitiot A, Richer L, et al. Sex differences in the growth of white matter during adolescence. NeuroImage 2009;45:1055–66. doi:10.1016/j.neuroimage.2009.01.023.
[38] Giedd JN, Blumenthal J, Jeffries NO, Castellanos FX, Liu H, Zijdenbos A, et al. Brain development during childhood and adolescence: a longitudinal MRI study. Nat Neurosci 1999;2:861–3. doi:10.1038/13158.
[39] Lebel C, Caverhill-Godkewitsch S, Beaulieu C. Age-related regional variations of the corpus callosum identified by diffusion tensor tractography. NeuroImage 2010;52:20–31. doi:10.1016/j.neuroimage.2010.03.072.
[40] Underhill HR, Yuan C, Yarnykh VL. Direct quantitative comparison between cross-relaxation imaging and diffusion tensor imaging of the human brain at 3.0 T. NeuroImage 2009;47:1568–78. doi:10.1016/j.neuroimage.2009.05.075.
[41] Schmithorst VJ, Wilke M, Dardzinski BJ, Holland SK. Correlation of white matter diffusivity and anisotropy with age during childhood and adolescence: a cross-sectional diffusion-tensor MR imaging study. Radiology 2002;222:212–8. doi:10.1148/radiol.2221010626.

[42] Yarnykh VL, Yuan C. Cross-relaxation imaging reveals detailed anatomy of white matter fiber tracts in the human brain. NeuroImage 2004;23:409–24. doi:10.1016/j.neuroimage.2004.04.029.

Table/Figure legends

Table 1 shows demographics within each site and the whole group. The statistics column tests for demographic differences between the two sites (Porto Alegre and Sao Paulo). At P <0.05, there was a significant difference related to gender.

Table 2. Correlation between FA and MD measures in 20 tracts: (i) FA/MD all measures showed a significant correlation at P level < 10^{-5}, except for forceps minor (FMn) and superior longitudinal fasciculus temporal part left (SLFtemp L) that showed no significant correlation; (ii) FA/axial diffusivity showing no significant correlation at P level < 0.05 for most tracts, except for SLFtemp right; (iii) FA/radial diffusivity all measures were significantly correlated at P level < 10^{-5}, except for forceps minor that showed no significant correlation; (iv) no significant correlation was found between FA and MTR.

Table 3. Age-effect GLM of mean values for 20 tracts according to JHU atlas (P level with post hoc Bonferroni correction).

Figure 1. FA showing positive correlation (yellow-red) with age in the TFCE analysis (P < 0.01; FWE correction). Mean, axial and radial diffusivities showing negative correlation (blue-light blue) with age in the TFCE analysis (P < 0.01; FWE corrected). The statistical maps are overlaid on the T1 anatomical image.

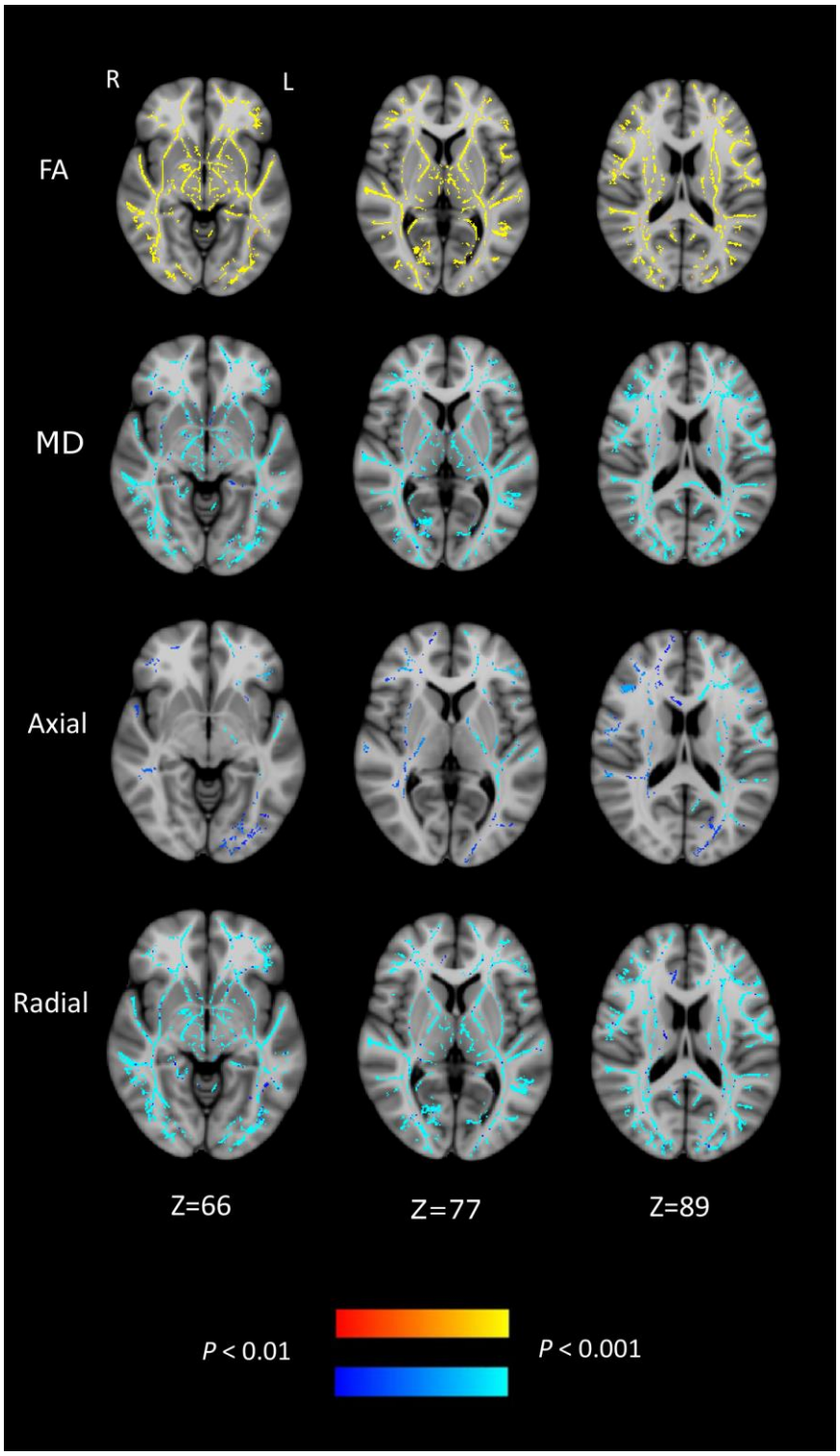
	Total Sample	**Porto Alegre**	**Sao Paulo**	**Statistics (PA vs SP)**
Sample size	n=176	n=86	n=90	---
Age mean (SD)	10.45 (2.31)	10.67 (2.64)	10.24 (1.95)	*P*=0.22
Gender (male/female)	88/88	50/36	38/52	*P*=0.03
IQ mean (SD)	103.15 (17.67)	104.97 (18.32)	101.42 (16.94)	*P*= 0.18

TRACT	FA/MD	FA/axial	FA/radial
antTHR L	-,54**	,17	-,77**
antTHR R	-,67**	-,22	-,79**
CS L	-,61**	,14	-,88**
CS R	-,68**	-,15	-,85**
CG L	-,52**	-,01	-,77**
CG R	-,50**	,03	-,72**
CGH L	-,68**	,05	-,85**
CGH R	-,56**	,03	-,77**
FMj	-,74**	-,09	-,84**
FMn	-,29*	,11	-,13
infFO L	-,56**	,16	-,83**
infFO R	-,63**	,01	-,83**
infLF L	-,56**	--	-,74**
infLF R	-,52**	,16	-,74**
SLF L	-,50**	-,01	-,74**
SLF R	-,65**	,17	-,83**
UF L	-,61**	-,09	-,80**
UF R	-,52**	,08	-,74**
SLFtemp L	-,20	,45**	-,37**
SLFtemp R	-,44**	,22	-,76**

$P < 10^{-5}$ **, $P = 0.001$* Spearman correlations (*P* values followed by Bonferroni post hoc correction)

Tracts	FA	MD	axial	radial	MTR
antTHR L	†	--	--	**	--
antTHR R	†	--	--	--	--
CS L	†	†	--	†	--
CS R	†	*	--	--	--
CG L	†	†	*	†	--
CG R	*	--	--	--	--
CGH L	*	--	--	--	--
CGH R	*	--	--	--	--
FMj	†	†	--	†	--
FMn	--	*	--	--	--
infFO L	†	†	--	†	--
infFO R	**	†	--	†	--
infLF L	†	†	--	†	--
infLF R	†	†	--	†	--
SLF L	†	†	†	†	--
SLF R	**	†	*	†	--
UF L	†	†	--	†	--
UF R	**	†	--	†	--
SLFtemp L	**	†	--	**	--
SLFtemp R	--	†	--	†	--

-- no significant finding, * p< .05, ** p< .01, † p< .001


R
L
FA
MD
Axial
Radial
Z=66
Z=77
Z=89
P < 0.01
P < 0.001

Supplementary material

Table S1. Demographics of the three age groups

Age groups	**7-8**	**9-11**	**12-14**	**Statistics**
Sample size	n=49	n=63	n=63	---
Gender (male/female)	22/27	33/30	33/31	
IQ mean (SD)	107.02 (17.74)	102.09 (16.33)	101.25 (18.67)	*P*= 0.19

Table S2. Spearman correlations between MTR and DTI measures. After Bonferroni post hoc correction, no significant correlations were found.

TRACT	MTR/FA	MTR/MD	MTR/axial	MTR/radial
antTHR L	-,02	--	,04	,03
antTHR R	--	-,06	-,06	-,02
CS L	-,08	,04	-,07	,08
CS R	,04	,03	,11	,05
CG L	-,01	-,01	,02	-,03
CG R	,15	-,18	-,08	-,19
CGH L	,01	-,01	,03	,01
CGH R	,05	-,01	,02	-,01
FMj	,18	-,16	,01	-,19
FMn	-,06	-,10	-,12	-,07
infFO L	,03	-,06	-,06	-,03
infFO R	,03	-,12	-,12	-,11
infLF L	--	-,04	-,04	-,05
infLF R	,04	-,01	,01	-,02
SLF L	,12	-,04	,05	-,08
SLF R	,14	-,22	-,15	-,21
UF L	-,06	-,07	,02	,07
UF R	--	-,07	-,09	-,05
SLFtemp L	-,01	-,03	,06	-,05
SLFtemp R	,11	-,02	,09	-,07